(a) contacting the sample with a capture reagent that is specific for a API-6; and

C2 cont'd

(b) detecting whether binding has occurred between the capture reagent and API-6 in the sample.

C3

63. **(Twice Amended)** The method of claim 60, wherein the capture reagent recognizes a post translational component part of API-6 which distinguishes said API-6 from other members of the NCAM gene family.

Please add the following claims 68 and 69:

~~72~~68. **(New)** The method of claim 59, wherein the API-6 is quantitatively detected.

C4

~~73~~69. **(New)** The method of claim 68, wherein the quantitatively detected API-6 is compared to a previously determined reference range or control.

Rule 1.126

Remarks

Claims 51-55 and 59-67 are currently under examination. Claims 59, 60 and 63 have been amended. New claims 68 and 69 have been added. Claims 51-55 have been cancelled. Support for the amendments may be found throughout the specification, including the claims as originally filed. No new matter has been added.

Amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite prosecution of the present application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Applicants respectfully request entry of these claim amendments, since these amendments present the claims in better form for consideration on appeal.

Election/Restrictions

Applicants acknowledge Examiner's restriction of the invention to a method for screening of Alzheimer's disease by detecting API-6 in a biological sample. However, Applicants respectfully point out that MPEP §2434 specifically states that "Nucleotide sequences encoding the same protein are not considered to be independent and distinct and will continue to

be examined together."(emphasis added). Applicants respectfully resubmit that API-3, API-6, API-47, API-58, API-145 and API-239 are peptides that comprise a single protein, NCAM (Accession No. 015179) and ought to be examined together. In particular, API-3 corresponds to SEQ ID NO: 14, API-6 corresponds to SEQ ID NOs: 36-39, API-47 corresponds to SEQ ID NOs: 1-3, API-58 corresponds to SEQ ID NOs: 58-62, API-145 corresponds to SEQ ID NOs: 412-414 and API-239 corresponds to SEQ ID NO: 267. Importantly, there is a significant overlap among these sequence fragments (See attached Exhibit A). Applicants respectfully note that there are over 300 API's disclosed in the specification, corresponding to multiple proteins that play a role in Alzheimer's disease. Applicants are solely attempting to claim the API's corresponding to the NCAM molecule of which there are six. Applicants respectfully maintain that the required search would not pose an undue burden on the Examiner as the six API's all correspond to one protein. Applicants respectfully request that the Examiner reconsider the restriction.

In any event, Applicants further reserve the option to further prosecute claims directed to NCAM and other proteins that are disclosed in the specification in a subsequent patent application.

Specification

Examiner objects to the embedded hyperlinks and/or other form of browser executable code. Applicants respectfully argue that §608.01 of the MPEP entitled "Hyperlinks and Other forms of Browser Executable Code in the Specification," specifically allows the use of hyperlinks "[w]here the hyperlinks and/or other forms of browser executable codes are part of the applicant's invention and it is necessary to have them included in the patent application in order to comply with the requirements of 35 U.S.C. 112, first paragraph, and applicant does not intend to have these hyperlinks be active links, examiners should not object to these hyperlinks." Applicants respectfully submit that hyperlinks included in the specification are necessary for the Applicants to comply with §112, first paragraph. For instance, http://www.ncbi.nlm.nih.gov on page 8, line 9 teaches a person skilled in the art how to determine percent identity between two sequences. Http://www.expasy.ch/ and www.mann.emblheidelberg.ce/Services/PeptideSearch/, page 24, lines 21-23, provides examples of methods and tools that allow a person skilled in the art to identify sequence information from proteins analyzed by mass spectroscopy and/or tandem

mass spectroscopy. Http://www.expasy.ch/ and http://www.ncbi.nlm.nih.gov, page 54, lines 20 and 25 provide a resource for a person skilled in the art to databases of protein sequences comprising the APIs in Tables IV and V. Http://www.toulouse.infra.fr/prodom.html and http://www.ch.embnet.org/software/TMPRED_form.html, page 75, lines 25 and 30, teach one skilled in the art how to identify individual domains of the disclosed APIs. Http://www.ncbi.nlm.nih.gov, on page 135, line 10, describes how Applicants analyzed proteins identified by the disclosed 2-D electrophoresis method. Finally, http://www.promega.com found in Table X on page 98 describes suitable assays for detecting or quantifying enzymatic or binding activity of an API. Applicants respectfully request that the Examiner reconsider her objection.

Examiner notes to the use of trademarks in the specification and requests that, whenever used trademarks should be both capitalized and accompanied with generic terminology. Applicants have amended the specification to correct the capitalization of the trademarks as shown below. Applicants respectfully submit that MPEP §608.01(v) states that trademarks are permissible in an application if "[I]n this country, their meanings are well known and satisfactorily defined in the literature." Applicants respectfully submit that the meanings of the trademarks used in this specification are both well known and defined in the scientific literature. Applicants respectfully request that the Examiner reconsider her objection.

Claim Objections

Examiner requires correction to claim 59, specifically, Examiner suggests that the term "administer" should be "administered." Claim 59 has been amended and the amendment is believed to obviate the objection. Reconsideration and withdrawal of the objection is respectfully requested.

Rejection of claims 51-55 and 59-67 under 35 U.S.C. §112, first paragraph

The Examiner states that claims 51-55 and 59-67 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully traverse the rejection.

Applicants have amended claim 59 to delete the following language "determining the stage or severity of Alzheimer's disease in a subject, identifying a subject at risk of developing

Alzheimer's disease, or monitoring the effect of therapy administer to a subject having Alzheimer's disease." However, Applicants wish to explicitly state that this deletion was only done for clarification purposes, as the term "diagnosis" is defined in the specification as "diagnosis, prognosis, monitoring, selecting participants in clinical trials, and identifying patients most likely to respond to a particular therapeutic treatment." The deletion is not in any way an indication on the part of the Applicants that breadth of the claim is being narrowed or that the subject matter deleted is not being pursued.

The Examiner contends that "the state of the prior art is such that there is no reference of record that would associate API-6, which is represented by SEQ ID Nos:36-39 with Alzheimer's disease or with neurodegeneration in general. The instant specification fails to provide any evidence or sound scientific reasoning that would support a conclusion that the decrease in presence of polypeptides of SEQ ID Nos:36-39 in a biological sample would have any association with Alzheimer's disease." Applicants respectfully submit that it is precisely the data presented in the specification that discloses an association between API-6 and Alzheimer's disease. Applicants additionally submit a 37 CFR 1.132 declaration by Dr. Holly Soares, a Senior Research Investigator at Pfizer and a Adjunct Assistant Professor in the Department of Anatomy and Neurobiology at Morehouse School of Medicine. The declaration presents further documentation that a decrease in the level of API-6 is associated with Alzheimer's disease. In particular, the declaration contains additional data showing that over a period of eight years a patient diagnosed with Alzheimer's disease exhibits a precipitous cognitive decline (as monitored by the mini mental state expansion test (MMSE)), a clinical marker of the disease, that is associated with a concomitant decrease in the level of API-6. Notably, these results are statistically significant. Applicants respectfully submit that the disclosure provided in the specification, along with the additional data presented in the declaration, unequivocally show that API-6 is associated with Alzheimer's disease. In view thereof, reconsideration and withdrawal of the 35 U.S.C. §112, first paragraph, rejection are respectfully requested.

The Examiner also states that "it was proposed that the detection of API-6 in a biological sample of a subject will implement the claimed method for screening, diagnosis or prognosis of Alzheimer's disease (AD), as well as for determining stage or severity of AD, for identifying subjects at risk for developing AD and for monitoring the progress and a treatment of AD." However, according to the Examiner, the instant specification "provides neither enough guidance for such a method of treatment, nor working examples, thus requiring undue experimentation on part of one skilled in the art to discover how to practice the claimed invention." Furthermore, the Examiner states that the specification does not provide sufficient guidance on "how to estimate what level of decrease of API-6 can be considered sufficient to be associated with AD."

Applicants respectfully submit that, in order to practice the claimed invention, one skilled in the art need only to detect a decrease in API-6 relative to a control sample or a reference range. All that is required to diagnose AD is the ability to detect a decrease in API-6 relative to a control sample or a reference range. Applicants also submit the data presented in the declaration in support of the assertion that that no undue experimentation is required to practice the invention as claimed. A decrease in the concentration of API-6 is detected as the disease progresses. Notably, the concentration of API-6 is decreased relative to the control range in every instance and it correlates with the progressive onset of the disease. It is evident that an absolute determination of the concentration of API-6 is not necessary to practice the claimed invention; what is required is a determination of whether there is a decreased level of API-6 relative to a control sample or a reference range.

Applicants maintain that the specification and the prior art is replete with teachings and guidance regarding available methods that may be used to determine whether there is a decreased level of API-6 relative to a control sample or a reference range. The specification discloses multiple methods that can be used to detect API-6, including, but not limited to: kinase assays, enzyme assays, binding assays, immunoassays and western blotting (page 39, lines 21-26). More detailed disclosure on each of those methods is provided on pages 39 to 48. Additionally, the specification discloses statistical techniques for identifying API's and API clusters (pages 48 to 50). Use of the invention in clinical studies are described on page 50, lines 10-24, and use of API's in the diagnosis of AD is described on page 90, line 24 to page 91, line 32. Applicants respectfully submit that no undue experimentation is needed to practice the claimed invention.

Examiner further states that the disclosure is not enabling because the specification does not disclose "what amino acid sequences from SEQ ID NOs: 36-39 are critical for the determination that API-6 is decreased." Applicants respectfully submit that SEQ ID NO's: 36-39 all derive from AF-21 which is defined by a single molecular weight and a single isoelectric point. AF-21 and, concomitantly, API-6 are all part of the same isoform of NCAM. As a result, SEQ ID NO's: 36-39 will all decrease uniformly with the onset and progression of AD. Similarly, because SEQ ID NO's: 36-39 are all part of the same isoform of NCAM, there would not be a situation where "one API is decreased and other API's are increased" as the Examiner suggests.

Finally, Examiner states that "it is clear from the results presented in Table I, page 12, that AF-21 (which corresponds to API-6) is only decreased in subjects with AD compare to normal subjects. Thus one of ordinary skill in the art would not reasonably conclude that detection of API-6 in a biological sample of a subject would associate such subject with any disease." Applicants have amended the language of the claims to clarify that it is the detection of

a decrease in API-6 that is associated with Alzheimer's disease. Applicants respectfully request that the Examiner withdraw the rejection.

Rejection of Claims 51-55 and 60-67 under 35 U.S.C. §112, second paragraph

Claims 51-55 and 60-67 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

Claim 51 is rejected as vague and indefinite for recitation of "Accession No. 15179." Claim 51 has been cancelled. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 63 is rejected as vague and ambiguous since it is not clear to the Examiner what "other members of the gene family" are encompassed within the claim. Claim 63 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claims 52-55, 60-62 and 64-67 are rejected as being indefinite for being dependent from indefinite claims. Claims 52-55 have been cancelled. Claims 60-63 and 64-67 have been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,
FOLEY HOAG LLP
155 Seaport Blvd.
Boston, MA 02109
Telephone: (617) 832-1000
Facsimile: (617) 832-7000
Date: April 28, 2003

Customer Number : 25181

Beth E. Arnold
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Registration No. 35,430

Copy of amended claims with changes marked thereon

In the specification:

Please amend the specification as set forth below:

Please replace the first paragraph of Section 6.1.15, page 134, line 27 through page 135, line 17, with the following paragraph:

Proteins in AFs were robotically excised and processed to generate tryptic digest peptides. Tryptic peptides were analyzed by mass spectrometry using a PerSeptive Biosystems Voyager- DE™ STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analyzed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupol Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a [nanoflow™] Nanoflow™ electrospray Z-spray source. For partial amino acid sequencing and identification of APIs uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptides returned from the database, and a mass increment for all Cys residues to account for carbamidomethylation. The database searched was database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at http://www.ncbi.nlm.nih.gov/. Following identification of proteins through spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no proteins could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell et al., 1992, Rapid Commun. Mass Spectrom. 6:658-662).

In the claims:

Pease amend claims 59-67 as set forth below:

59. **(Twice Amended)** A method for screening, diagnosis, or prognosis of Alzheimer's disease in a subject, [determining the stage or severity of Alzheimer's disease in a subject, identifying a subject at risk of developing Alzheimer's disease, or monitoring the effect of therapy administer to a subject having Alzheimer's disease,] the method comprising [quantitatively] detecting, in a biological sample, [at least one of the Alzheimer's disease-Associated Protein Isoforms (APIs): API-3,] API-6, [API-47, API-58, API-239,] wherein a decreased level of said API-6 [(s)], relative to a control sample or a reference range, indicates the presence or degree of Alzheimer's disease or a subject at risk of developing Alzheimer's disease.

60. **(Twice Amended)** The method of claim 59 [51,] wherein the step of detecting comprises:

(a) contacting the sample with a capture reagent that is specific for [a pre-selected] API-6; and

(b) detecting whether binding has occurred between the capture reagent and [at least one] API-6 in the sample.

61. (Previously amended) The method of claim 60, wherein step (b) comprises detecting the captured API using a directly or indirectly labeled detection reagent.

62. (Previously amended) The method of claim 60, wherein the biological sample is cerebrospinal fluid.

63. **(Twice Amended)** The method of claim 60, wherein the capture reagent recognizes a post translational component part of [the] API-6 which distinguishes [the] said API-6 from other members of the NCAM gene family.

Please add the following claims 68 and 69:

68. **(New)** The method of claim 59, wherein the API-6 is quantitatively detected.

69. **(New)** The method of claim 68, wherein the quantitatively detected API-6 is compared to a previously determined reference range or control.